WHAT IS CLAIMED:

1. A method for identifying one or more low abundance sequences differing by one or more single-base changes, insertions, or deletions, from a high abundance sequence in a plurality of target nucleotide sequences comprising:

providing a sample potentially containing one or more low abundance target nucleotide sequences with at least one sequence difference each from the high abundance target sequences;

providing a primary oligonucleotide primer set characterized by (a) a first oligonucleotide primer containing a target-specific portion, and (b) a second oligonucleotide primer containing a target-specific portion, wherein the primary oligonucleotide primers are suitable for hybridization on complementary strands of a corresponding high and low abundance target nucleotide sequences to permit formation of a polymerase chain reaction product, but have a mismatch which interferes with formation of such a polymerase chain reaction product when hybridized to any other nucleotide sequence present in the sample;

providing a polymerase;

blending the sample, the primary oligonucleotide primers, and the polymerase to form a primary polymerase chain reaction mixture;

subjecting the primary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the target-specific portions of the primary oligonucleotide primers hybridize to the target nucleotide sequences, and an extension treatment, wherein the hybridized primary oligonucleotide primers are extended to form primary extension products complementary to the target nucleotide sequence to which the primary oligonucleotide primer is hybridized;

providing a secondary oligonucleotide primer set characterized by (a) a first oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-

specific portion, wherein the secondary oligonucleotide primers in a particular set are suitable for hybridization on complementary strands of the primary extension products to permit formation of a secondary polymerase chain reaction product which contains or creates a restriction endonuclease recognition site when amplifying the high abundance target, but does not contain or create a restriction endonuclease recognition site when amplifying the one or more low abundance targets;

providing a polymerase;

blending the primary extension products, the secondary oligonucleotide primers, and the polymerase to form a secondary polymerase chain reaction mixture;

subjecting the secondary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the secondary oligonucleotide primers hybridize to the primary extension products, an extension treatment, wherein the hybridized secondary oligonucleotide primers are extended to form secondary extension products complementary to the primary extension products, wherein high abundance secondary extension products contain a restriction site but low abundance secondary extension products do not;

providing a restriction endonuclease;

blending the secondary extension product and the restriction endonuclease to form an endonuclease digestion reaction mixture;

subjecting the endonuclease digestion reaction mixture to an endonuclease digestion reaction such that the restriction endonuclease recognizes and cleaves the restriction endonuclease recognition site contained within or created when amplifying the high abundance target but not the low abundance target in the secondary extension products, thus selectively destroying the high abundance secondary extension products;

providing a tertiary oligonucleotide primer set characterized by (a) a first tertiary primer containing the same sequence as the 5' upstream portion of the first oligonucleotide primer of the secondary oligonucleotide primer set, and (b) a second tertiary primer containing the same sequence as the 5' upstream

portion of a second oligonucleotide primer of the secondary oligonucleotide primer set, wherein the set of tertiary oligonucleotide primers may be used to amplify all of the secondary extension products;

blending the secondary extension products, the tertiary oligonucleotide primer set, and the polymerase to form a tertiary polymerase chain reaction mixture;

subjecting the tertiary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the tertiary oligonucleotide primers hybridize to the secondary extension products, an extension treatment, wherein the hybridized tertiary oligonucleotide primers are extended to form tertiary extension products complementary to the secondary extension products;

providing a plurality of oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe, having a tertiary extension product-specific portion and a detectable reporter label, and (b) a second oligonucleotide probe, having a tertiary extension product-specific portion, wherein the oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a complementary tertiary extension product-specific portion, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample;

providing a ligase;

blending the tertiary extension product, the plurality of oligonucleotide probe sets, and the ligase to form a ligase detection reaction mixture;

subjecting the ligase detection reaction mixture to one or more ligase detection reaction cycles comprising a denaturation treatment, wherein any hybridized oligonucleotides are separated from the tertiary extension products, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base-specific manner to their respective tertiary extension products, if present, and ligate to one another to form a ligation product sequence containing (a) the detectable reporter label and (b) the tertiary extension product-

specific portions connected together, wherein the oligonucleotide probe sets may hybridize to nucleotide sequences other than their respective complementary tertiary extension products but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment; and

detecting the reporter labels of the ligation product sequences, thereby indicating the presence of one or more low abundance target nucleotide sequences in the sample.

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2. A method according to claim 1, wherein the oligonucleotide probes in a particular oligonucleotide probe set have a unique length so that the ligation product sequences which they form can be distinguished from other nucleic acids:

separating the ligation product sequences by electrophoretic mobility prior to said detecting and

distinguishing, after said detecting, the ligation product sequences which differ in electrophoretic mobility.

3. A method according to claim 1, wherein the second oligonucleotide probe of each oligonucleotide probe set has an addressable array-specific portion, said method further comprising:

providing a solid support with different capture oligonucleotides immobilized at different particular sites, wherein the capture oligonucleotides have nucleotide sequences complementary to the addressable array-specific portions and

contacting the ligase detection reaction mixture, after said subjecting it to one or more ligase detection reaction cycles, with the solid support under conditions effective to hybridize the ligation product sequences to the capture oligonucleotides in a base-specific manner, thereby capturing the addressable array-specific portions to the solid support at the site with the complementary capture oligonucleotide, wherein said detecting indicates the presence of ligation product sequences captured using the addressable array-specific portions and immobilized to the solid support at particular sites, thereby indicating the presence of one or more target nucleotide sequences in the sample.

4. A method according to claim 1, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts with one or more high abundance sequence in a plurality of target nucleotides, are quantified, said method further comprising:

quantifying, after said subjecting the primary polymerase chain reaction mixture to one or more polymerase chain reaction cycles, the amounts of primary extension products;

providing a known amount of one or more marker target nucleotide sequences;

providing one or more sequence-specific probe sets, including probe sets specifically designed for the marker target nucleotide sequences;

blending the marker target nucleotide sequences, and the probe sets specifically designed for the marker target nucleotide sequences with ligase detection reaction mixture;

quantifying the amount of ligation product sequences; and

comparing the amount of ligation product sequences generated from the unknown low abundance sample with the amount of ligation product sequences generated from known amounts of marker target nucleotide sequences to provide a quantitative measure of the relative level of one or more low abundance target nucleotide sequences in the sample.

5. A method according to claim 4, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts is present in less than a 1:1,000 molar ratio to the amount of the high abundance sequence in a plurality of target nucleotides.

6. A method according to claim 4, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a

sample in unknown amounts is present in less than a 1:10,000 molar ratio to the amount of the high abundance sequence in the sample.

7. A method according to claim 4, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts is present in less than a 1:100,000 molar ratio to the amount of the high abundance sequence in the sample.

8. A method according to claim 1, wherein the efficiency and accuracy of converting the high abundance primary polymerase chain reaction product into a secondary polymerase chain reaction product containing a restriction endonuclease site is improved by performing the following step prior to the providing the secondary oligonucleotide primer set:

providing a pre-secondary oligonucleotide primer set characterized by (a) a first oligonucleotide primer, having a target-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion, wherein the target-specific portions are identical or substantially identical to the secondary oligonucleotide primer set but at least one primer contains one or more nucleotide analogs, wherein the oligonucleotide primers in a particular set are suitable for hybridization on complementary strands of the primary extension products to permit formation of a pre-secondary polymerase chain reaction product which contains one or more nucleotide analogs and opposite strand base changes, wherein the pre-secondary oligonucleotide primer set facilitates conversion of the primary polymerase chain reaction product sequence into a restriction endonuclease recognition site in the subsequent secondary polymerase chain reaction;

providing a polymerase;

blending the primary extension products, the pre-secondary oligonucleotide primers, and the polymerase to form a pre-secondary polymerase chain reaction mixture;

subjecting the secondary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment,

wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the secondary oligonucleotide primers hybridize to the primary extension products, an extension treatment, wherein the hybridized secondary oligonucleotide primers are extended to form pre-secondary extension products complementary to the primary extension products, wherein the pre-secondary extension products contain one or more nucleotide analogues and opposite strand base changes which facilitate conversion of the primary polymerase chain reaction product sequence into a restriction endonuclease recognition site in the subsequent secondary polymerase chain reaction, wherein the pre-secondary extension products are then used in place of the primary extension products in the secondary polymerase chain reaction mixture.

9. A method according to claim 8, wherein the oligonucleotide probes in a particular oligonucleotide probe set have a unique length so that the ligation product sequences which they form can be distinguished from other nucleic acids:

separating the ligation product sequences by electrophoretic mobility prior to said detecting and

distinguishing, after said detecting, the ligation product sequences which differ in electrophoretic mobility.

10. A method according to claim 8, wherein the second oligonucleotide probe of each set has an addressable array-specific portion, said method further comprising:

providing a solid support with different capture oligonucleotides immobilized at different particular sites, wherein the capture oligonucleotides have nucleotide sequences complementary to the addressable array-specific portions and

contacting the ligase detection reaction mixture, after said subjecting it to one or more ligase detection reaction cycles, with the solid support under conditions effective to hybridize the ligation product sequences to the capture oligonucleotides in a base-specific manner, thereby capturing the addressable array-specific portions to the solid support at the site with the

complementary capture oligonucleotide, wherein said detecting indicates the presence of ligation product sequences captured using the addressable array-specific portions and immobilized to the solid support at particular sites, thereby indicating the presence of one or more target nucleotide sequences in the sample.

11. A method according to claim 8, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts with one or more high abundance sequence in a plurality of target nucleotide, are quantified, said method further comprising:

quantifying, after said subjecting the primary polymerase chain reaction mixture to one or more polymerase chain reaction cycles, the amounts of primary extension products;

providing a known amount of one or more marker target nucleotide sequences;

providing one or more sequence-specific probe sets, including probe sets specifically designed for the marker target nucleotide sequences;

blending the marker target nucleotide sequences, and the probe sets specifically designed for the marker target nucleotide sequences with ligase detection reaction mixture;

quantifying the amount of ligation product sequences; and

comparing the amount of ligation product sequences generated from the unknown low abundance sample with the amount of ligation product sequences generated from known amounts of marker target nucleotide sequences to provide a quantitative measure of the relative level of one or more low abundance target nucleotide sequences in the sample.

12. A method according to claim 11, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts is present in a molar ratio of than less than 1:1,000 to the amount of the high abundance sequence in the sample.

13. A method according to claim 11, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts is present in a molar ratio of less than 1:10,000 to the amount of the high abundance sequence in the sample.

14. A method according to claim 11, wherein the relative amounts of one or more of a low abundance sequence, differing by one or more single-base changes, insertions, deletions, or translocations and present in a sample in unknown amounts is present in a ratio molar ratio of less than 1:100,000 to the amount of the high abundance sequence in the sample.

15. A method according to claim 8, where the nucleotide analog of at least one oligonucleotide primer of the pre-secondary oligonucleotide primer set is at the 3' end of the primer.

16. A method according to claim 8, where the nucleotide analog is selected from the group consisting of 1-(2′-deoxy-ß-D-ribofuranosyl)imidazole-4-carboxamide, 1-(2′-deoxy-ß-D-ribofuranosyl)-3-nitropyrrole, 2'-deoxyinosine, 6-(2′-deoxy-ß-D-ribofuranosyl)-6H,8H-3,4-dihydropyrimido[4,5-c][1,2]oxazine-7-one, 2-amino-7-(2′-deoxy-ß-D-ribofuranosyl)-6-methoxyaminopurine, 1-(2′-deoxy-ß-D-ribofuranosyl)-4-iodopyrazole, 1-(2′-deoxy-ß-D-ribofuranosyl)pyrrole-3-carboxamide, and 1-(2′-deoxy-ß-D-ribofuranosyl)-4-nitropyrazole.

17. A method according to claim 1 further comprising:

repeating the endonuclease digestion reaction after said subjecting the tertiary polymerase chain reaction mixture to two or more polymerase chain reaction cycles and after said subjecting the ligase detection reaction mixture to one or more ligase detection reaction cycles, wherein, during said repeating the endonuclease digestion reaction, the restriction endonuclease recognizes and cleaves the restriction endonuclease recognition site contained within any

remaining high abundance target, thereby selectively destroying the high abundance tertiary extension products.

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18. A kit for identifying one or more low abundance sequences differing by one or more single-base changes, insertions, or deletions, from a high abundance sequence in a plurality of target nucleotide sequences comprising:

providing a primary oligonucleotide primer set characterized by (a) a first oligonucleotide primer containing a target-specific portion, and (b) a second oligonucleotide primer containing a target-specific portion, wherein the primary oligonucleotide primers are suitable for hybridization on complementary strands of a corresponding high and low abundance target nucleotide sequences to permit formation of a primary extension product, but have a mismatch which interferes with formation of such a polymerase chain reaction product when hybridized to any other nucleotide sequence present in the sample;

providing a secondary oligonucleotide primer set characterized by (a) a first oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific portion, wherein the secondary oligonucleotide primers in a particular set are suitable for hybridization on complementary strands of the primary extension products to permit formation of a secondary extension product which contains or creates a restriction endonuclease recognition site when amplifying the high abundance target, but does not contain or create a restriction endonuclease recognition site when amplifying the one or more low abundance targets;

providing a tertiary oligonucleotide primer set characterized by (a) a first tertiary primer containing the same sequence as the 5' upstream portion of the first oligonucleotide primer of the secondary oligonucleotide primer set, and (b) a second tertiary primer containing the same sequence as the 5' upstream portion of a second oligonucleotide primer of the secondary oligonucleotide primer set, wherein the set of tertiary oligonucleotide primers may be used to amplify all of the secondary extension products; and

providing a plurality of oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe, having a tertiary extension

product-specific portion and a detectable reporter label, and (b) a second oligonucleotide probe, having a tertiary extension product-specific portion, wherein the oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a complementary tertiary extension product-specific portion, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample.

19. A kit according to claim 18 further comprising:
providing a pre-secondary oligonucleotide primer set characterized by (a) a first oligonucleotide primer, having a target-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion, wherein the target-specific portions are identical or substantially identical to the secondary oligonucleotide primer set but at least one primer contains one or more nucleotide analogs, wherein the pre-secondary oligonucleotide primers in a particular set are suitable for hybridization on complementary strands of the primary extension products to permit formation of a pre-secondary extension product which contains one or more nucleotide analogs and opposite strand base changes, wherein the pre-secondary oligonucleotide primer set facilitates conversion of the primary extension product sequence into a restriction endonuclease recognition site in a subsequent secondary polymerase chain reaction.

20. A kit according to claim 18 further comprising:
a ligase.

21. A kit according to claim 18 further comprising:
a polymerase.

22. A kit according to claim 18 further comprising:
a restriction endonuclease.